USER GUIDE

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TOPO® TA Cloning® Kit

Five-minute cloning of *Taq* polymerase-amplified PCR products

Catalog numbers (pCR™2.1-TOPO® vector) K4500-01, K4500-40, K4500-J10, K4510-20, K4520-01, K4520-40, K4550-01, K4550-40, K4560-01, K4560-40, K4500-02, K4510-22, 450641

Catalog numbers (pCR™II-TOPO® vector) K4600-01, K4600-J10, K4600-40, K4610-20, K4620-01, K4620-40, K4650-01, K4650-40, K4660-01, K4660-40

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INFORMATION FOR EUROPEAN CUSTOMERS

The Mach1™-T1^{R} *E. coli* strain is genetically modified to carry the *lac*ZΔM15 *hsd*R *lac*X74 *rec*A *end*A *ton*A genotype. As a condition of sale, this product must be in accordance with all applicable local legislation and guidelines including EC Directive 90/219/EEC on the contained use of genetically modified organisms.

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About this guide

IMPORTANT!

Before using this product, read and understand the information in the "Safety" appendix in this document.

Changes from previous version

Revision	Date	Description
A.0	24 February 2014	• Increase from 20 to 25 reaction kit size. • Include Cat. nos. K4500-J10 & K4600-J10 • Version numbering changed to alphanumeric format and reset to A in conformance with internal document control procedures.

Product information

Contents and storage

Shipping and storage

TOPO® TA Cloning® Kits are shipped on dry ice. Kits containing competent cells contain a box with TOPO® TA Cloning® reagents (Box 1) and a box with One Shot® Chemically Competent or Electrocomp™ cells (Box 2).

TOPO® TA Cloning® Kits supplied with the PureLink® Quick Plasmid Miniprep Kit (Cat. nos. K4500-02 and K4510-02) are shipped with an additional box containing reagents for plasmid purification (Box 3).

TOPO® TA Cloning® Kit for Subcloning (Cat. no. 450641) is shipped with only the TOPO® TA Cloning® reagents (Box 1).

Box	Store at
1	–30°C to –10°C in a non-frost-free freezer
2	–85°C to –68°C
3	Room temperature (15°C to 30°C)

Continued on next page

Contents and storage, continued

Types of TOPO® TA Cloning® Kits

TOPO® TA Cloning® Kits are available with pCR™ 2.1-TOPO® or pCR™ II-TOPO® vector. Kits with competent cells are available with One Shot® Chemically or Electrocomp™ competent cells as described in the following table (see page 8 for the genotypes of the strains).

Note: Cat. no. 450641 is not supplied with competent cells.

Select TOPO® TA Cloning® Kits are also available with PureLink® Quick Plasmid Miniprep Kit.

Product	Cat. no.	One Shot® Cells	Type of Cells	Reactions
TOPO® TA Cloning® Kit (with pCR™2.1-TOPO® vector)	K4500-01	TOP10	chem. competent	25
	K4500-40	TOP10	chem. competent	50
	K4500-J10	TOP10	chem. competent	10
	K4510-20	Mach1™-T1^R	chem. competent	25
	K4520-01	DH5α™-T1^R	chem. competent	25
	K4520-40	DH5α™-T1^R	chem. competent	50
	K4550-01	TOP10F´	chem. competent	25
	K4550-40	TOP10F´	chem. competent	50
	K4560-01	TOP10	electrocompetent	25
	K4560-40	TOP10	electrocompetent	50
	450641	Not supplied	NA	25
TOPO® TA Cloning® Kit (with pCR™2.1-TOPO® vector and PureLink® Quick Plasmid Miniprep Kit)	K4500-02	TOP10	chem. competent	25
	K4510-02	Mach1™-T1^R	chem. competent	25
TOPO® TA Cloning® Kit Dual Promoter (with pCR™II-TOPO® vector)	K4600-01	TOP10	chem. competent	25
	K4600-40	TOP10	chem. competent	50
	K4600-J10	TOP10	chem. competent	10
	K4610-20	Mach1™-T1^R	chem. competent	25
	K4620-01	DH5α™-T1^R	chem. competent	25
	K4620-40	DH5α™-T1^R	chem. competent	50
	K4650-01	TOP10F´	chem. competent	25
	K4650-40	TOP10F´	chem. competent	50
	K4660-01	TOP10	electrocompetent	25
	K4660-40	TOP10	electrocompetent	50

Continued on next page

TOPO® TA Cloning® reagents

TOPO® TA Cloning® reagents (Box 1) are listed in the following table. Note that the user must supply *Taq* polymerase. Store Box 1 at –30°C to –10°C.

Item	Concentration	Amount		
		10 Rxns	25 Rxns	50 Rxns
pCR™2.1-TOPO® vector ***or*** pCR™II-TOPO® vector	10 ng/µL plasmid DNA in: 50% glycerol 50 mM Tris-HCl, pH 7.4 (at 25°C) 1 mM EDTA 1 mM DTT 0.1% Triton X-100 100 µg/mL BSA phenol red	10 µL	25 µL	2 × 25 µL
10X PCR Buffer	100 mM Tris-HCl, pH 8.3 (at 42°C) 500 mM KCl 25 mM $MgCl_2$ 0.01% gelatin	100 µL	100 µL	2 × 100 µL
Salt Solution	1.2 M NaCl 0.06 M $MgCl_2$	50 µL	50 µL	2 × 50 µL
dNTP Mix	12.5 mM dATP; 12.5 mM dCTP 12.5 mM dGTP; 12.5 mM dTTP neutralized at pH 8.0 in water	10 µL	10 µL	2 × 10 µL
M13 Forward (–20) Primer	0.1 µg/µL in TE Buffer	20 µL	20 µL	2 × 20 µL
M13 Reverse Primer	0.1 µg/µL in TE Buffer	20 µL	20 µL	2 × 20 µL
Control Template	0.1 µg/µL in TE Buffer	10 µL	10 µL	2 × 10 µL
Control PCR Primers	0.1 µg/µL each in TE Buffer	10 µL	10 µL	2 × 10 µL
Water	—	1 mL	1 mL	2 × 1 mL

Sequence of primers

The following table describes the sequence and pmoles supplied of the sequencing primers included in this kit.

Primer	Sequence	pMoles Supplied
M13 Forward (–20)	5´-GTAAAACGACGGCCAG-3´	407
M13 Reverse	5´-CAGGAAACAGCTATGAC-3´	385

PureLink® Quick Plasmid Miniprep Kit

For kit components of the PureLink® Quick Plasmid Miniprep Kit (Box 3) supplied with Cat. nos. K4510-02 and K4500-02 refer to the manual supplied with the miniprep kit.

Continued on next page

One Shot® reagents

The following table describes the items included in each One Shot® competent cells kit. Store at −85°C to −68°C.

Item	Composition	Amount		
		10 Rxns	25 Rxns	50 Rxns
S.O.C. Medium (may be stored at 4°C or room temperature)	2% Tryptone 0.5% Yeast Extract 10 mM NaCl 2.5 mM KCl 10 mM $MgCl_2$ 10 mM $MgSO_4$ 20 mM glucose	6 mL	6 mL	2 × 6 mL
TOP10, Mach1™-T1^R, DH5α™-T1^R, or TOP10F´ ***or*** TOP10 cells	Chemically competent Electrocomp™	11 × 50 µL	26 × 50 µL	2 × (26 × 50 µL)
pUC19 Control DNA	10 pg/µL	50 µL	50 µL	2 × 50 µL

Genotypes of E. coli strains

DH5α™-T1^R: Use this strain for general cloning and blue/white screening without IPTG. Strain is resistant to T1 bacteriophage.

F$^-$ ϕ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F)U169 *rec*A1 *end*A1 *hsd*R17(r_k^-, m_k^+) *pho*A *sup*E44 *thi*-1 *gyr*A96 *rel*A1 *ton*A (confers resistance to phage T1)

Mach1™-T1^R: Use this strain for general cloning and blue/white screening without IPTG. Strain is resistant to T1 bacteriophage.

F$^-$ ϕ80(*lac*Z)ΔM15 Δ*lac*X74 *hsd*R(r_k^-, m_k^+) Δ*recA*1398 *end*A1 *ton*A (confers resistance to phage T1)

TOP10: Use this strain for general cloning and blue/white screening without IPTG.

F$^-$ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L (Str^R) *end*A1 *nup*G

TOP10F´: This strain over expresses the Lac repressor (*lac*I^q gene). For blue/white screening, you will need to add IPTG to the plates to obtain expression from the *lac* promoter. This strain contains the F episome and can be used for single-strand rescue of plasmid DNA containing an f1 origin.

F´ {*lac*I^q Tn*10* (Tet^R)} *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L (Str^R) *end*A1 *nup*G

Information for non-U.S. customers using Mach1™-T1^R cells

The parental strain of Mach1™-T1^R *E. coli* is the non-K-12, wild-type W strain (ATCC #9637, S. A. Waksman). Although the parental strain is generally classified as Biosafety Level 1 (BL-1), we recommend that you consult the safety department of your institution to verify the Biosafety Level.

Description of the system

TOPO® TA Cloning®

TOPO® TA Cloning® provides a highly efficient, 5-minute, one-step cloning strategy ("TOPO® Cloning") for the direct insertion of *Taq* polymerase-amplified PCR products into a plasmid vector. No ligase, post-PCR procedures, or PCR primers containing specific sequences are required.

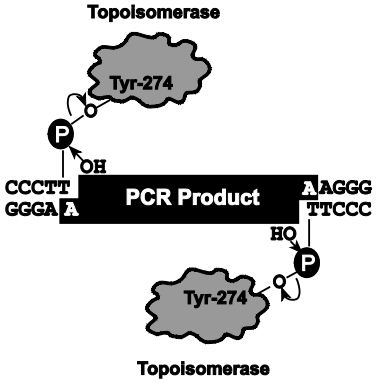
How Topoisomerase I works

The plasmid (pCR™II-TOPO® vector or pCR™2.1-TOPO® vector) is supplied linearized with:

- Single 3´-thymidine (T) overhangs for TA Cloning®
- Topoisomerase I covalently bound to the vector (referred to as "activated" vector)

Taq polymerase has a nontemplate-dependent terminal transferase activity that adds a single deoxyadenosine (A) to the 3´ ends of PCR products. The linearized vector supplied in this kit has single, overhanging 3´ deoxythymidine (T) residues. This allows PCR inserts to ligate efficiently with the vector.

Topoisomerase I from *Vaccinia* virus binds to duplex DNA at specific sites and cleaves the phosphodiester backbone after 5′-CCCTT in one strand (Shuman, 1991). The energy from the broken phosphodiester backbone is conserved by formation of a covalent bond between the 3′ phosphate of the cleaved strand and a tyrosyl residue (Tyr-274) of topoisomerase I. The phospho-tyrosyl bond between the DNA and enzyme can subsequently be attacked by the 5′ hydroxyl of the original cleaved strand, reversing the reaction and releasing topoisomerase (Shuman, 1994).



Experimental outline

- Produce your PCR product
- Set up the TOPO® cloning reaction (mix together the PCR Product and TOPO® vector)
- Incubate for 5 minutes at room temperature
- Transform the TOPO® cloning reaction into One Shot® Competent Cells or equivalent
- Select and analyze 10 white or light blue colonies for insert

Methods

Produce PCR products

Introduction

It is important to properly design your PCR primers to ensure that you obtain the product you need for your studies. After deciding on a PCR strategy and synthesizing the primers, you are ready to produce your PCR product.
Remember that your PCR product will have single 3´ adenine overhangs.

Note

Do not add 5´ phosphates to your primers for PCR. The PCR product synthesized will not ligate into pCR™2.1-TOPO® vector or pCR™II-TOPO® vector.

Materials supplied by the user

- *Taq* polymerase
- Thermocycler
- DNA template and primers for PCR product

Polymerase mixtures

If you wish to use a mixture containing *Taq* polymerase and a proofreading polymerase, *Taq* must be used in excess of a 10:1 ratio to ensure the presence of 3´ A-overhangs on the PCR product.

If you use polymerase mixtures that do not have enough *Taq* polymerase or a proofreading polymerase only, add 3' A-overhangs using the method on page 27.

Produce PCR products

1. Set up the following 50 µL PCR reaction. Use less DNA if you are using plasmid DNA as a template and more DNA if you are using genomic DNA as a template. Use the cycling parameters suitable for your primers and template. Be sure to include a 7–30 minute extension at 72°C after the last cycle to ensure that all PCR products are full length and 3´ adenylated.

DNA Template	10–100 ng
10X PCR Buffer	5 µL
50 mM dNTPs	0.5 µL
Primers (100–200 ng each)	1 µM each
Water	add to a final volume of 49 µL
Taq Polymerase (1 unit/µL)	1 µL
Total Volume	50 µL

2. Check the PCR product by agarose gel electrophoresis. You should see a single, discrete band. If you do not see a single band, refer to the following **Note**.

Note

If you do not obtain a single, discrete band from your PCR, you may gel-purify your fragment before using the TOPO® TA Cloning® Kit (see page 25). Take special care to avoid sources of nuclease contamination. Alternatively, you may optimize your PCR to eliminate multiple bands and smearing (Innis *et al.*, 1990). The PCR Optimizer™ Kit (see page 31) incorporates many of the recommendations found in this reference.

Perform the TOPO® Cloning reaction

Introduction

Once you have produced the desired PCR product, you are ready to TOPO®-clone it into the pCR™2.1-TOPO® or pCR™II-TOPO® vector and transform the recombinant vector into competent *E. coli*. It is important to have everything you need set up and ready to use to ensure that you obtain the best possible results. We suggest that you read this section and the sections detailing transformation of competent cells (pages 14–18) before beginning. If this is the first time you have TOPO®-cloned, perform the control reactions on pages 22–23 in parallel with your samples.

Note

We have found that including salt (200 mM NaCl; 10 mM $MgCl_2$) in the TOPO® Cloning reaction increases the number of transformants 2- to 3-fold. We have also observed that in the presence of salt, incubation times of greater than 5 minutes can also increase the number of transformants. This is in contrast to earlier experiments **without salt** where the number of transformants decreases as the incubation time increases beyond 5 minutes.

Inclusion of salt allows for longer incubation times because it prevents topoisomerase I from rebinding and potentially nicking the DNA after ligating the PCR product and dissociating from the DNA. The result is more intact molecules leading to higher transformation efficiencies.

Using salt solution in the TOPO® Cloning reaction

Because of the above results, we recommend adding salt to the TOPO® Cloning reaction. A stock salt solution is provided in the kit for this purpose. **Note that you must dilute the TOPO® Cloning reaction before transforming electrocompetent cells (see the following sections).** Read the following information carefully.

- For TOPO® Cloning and transformation into chemically competent *E. coli*, adding sodium chloride and magnesium chloride to a final concentration of 200 mM NaCl, 10 mM $MgCl_2$ in the TOPO® Cloning reaction increases the number of colonies over time. A Salt Solution (1.2 M NaCl; 0.06 M $MgCl_2$) is provided to adjust the TOPO® Cloning reaction to the recommended concentration of NaCl and $MgCl_2$.
- For TOPO® Cloning and transformation of electrocompetent *E. coli*, salt must also be included in the TOPO® Cloning reaction, but the amount of salt **must be reduced** to 50 mM NaCl, 2.5 mM $MgCl_2$ in order to prevent arcing. After performing the TOPO® Cloning reaction, and prior to electroporation, dilute the reaction 4-fold to achieve the proper salt concentration.

Continued on next page

Perform the TOPO® Cloning reaction, continued

Set Up the TOPO® Cloning reaction

The following table describes how to set up your TOPO® Cloning reaction (6 µL) for eventual transformation into either chemically competent or electrocompetent TOP10 or chemically competent DH5α™-T1^R, Mach1™-T1^R, or TOP10F' One Shot® *E. coli*. Additional information on optimizing the TOPO® Cloning reaction for your needs can be found on page 21.

Note: The red color of the TOPO® vector solution is normal and is used to visualize the solution.

Reagent*	Volume
Fresh PCR product	0.5–4 µL
Salt Solution	1 µL
Water	add to a total volume of 5 µL
TOPO® vector	1 µL
Final Volume	**6 µL**

*Store all reagents at –20°C when finished. Salt solutions and water can be stored at room temperature or 4°C.

Perform the TOPO® Cloning reaction

1. Mix the reaction gently and incubate for **5 minutes** at room temperature (22–23°C).

   Note: For most applications, 5 minutes will yield sufficient colonies for analysis. Depending on your needs, the length of the TOPO®-cloning reaction can be varied from 30 seconds to 30 minutes. For routine subcloning of PCR products, 30 seconds may be sufficient. For large PCR products (greater than 1 kb) or if you are TOPO®-cloning a pool of PCR products, increasing the reaction time will yield more colonies.
2. Place the reaction on ice and proceed to **Select a One Shot® chemical transformation protocol** on page 13.

   Note: You may store the TOPO® Cloning reaction at –20°C overnight.

Note

TOPO® TA Cloning® Kits are optimized to work with One Shot® Competent *E. coli* available from Life Technologies™. Use of other competent cells may require further optimization.

Performing the control TOPO® Cloning reaction is recommended as this control when used with the supplied protocol will demonstrate high cloning efficiencies.

Additionally, transforming a control plasmid is highly recommended to confirm transformation efficiencies when using alternative competent cells not supplied by Life Technologies.

Transform One Shot® competent cells

Introduction

After performing the TOPO® Cloning reaction, transform your pCR™2.1-TOPO® or pCR™II-TOPO® construct into the competent *E. coli*.

General guidelines for transformation are provided below. For transformation into competent *E. coli* supplied with your kit, refer to **Transform One Shot® Mach1™-T1^R competent cells** (pages 14–15) or **Transform One Shot® DH5α™-T1^R, TOP10, and TOP10F´ competent cells** (pages 16–18) depending on the competent *E. coli* you wish to transform.

To transform another competent strain, refer to the manufacturer's instructions.

Select a One Shot® chemical transformation protocol

Two protocols are provided to transform One Shot® chemically competent *E. coli*. Consider the following factors when choosing the protocol that best suits your needs.

If you wish to...	Then use the...
maximize the number of transformants	regular chemical transformation protocol
clone large PCR products (greater than 1000 bp)	
use kanamycin as the selective agent (see the following IMPORTANT!)	
obtain transformants as quickly as possible	rapid chemical transformation protocol

IMPORTANT!

If you will be using kanamycin as the selective agent for chemical transformation, use the regular chemical transformation protocol. The rapid chemical transformation protocol is only suitable for transformations using ampicillin selection.

Recommendation

If you use a plasmid template for your PCR that carries either the ampicillin or kanamycin resistance marker, we recommend that you use the other selection agent to select for transformants. For example, if the plasmid template contains the ampicillin resistance marker, then use kanamycin to select for transformants. The template is carried over into the TOPO® Cloning and transformation reactions, resulting in transformants that are ampicillin resistant and white, but are not the desired construct.

Transform One Shot® Mach1™-T1^R competent cells

Introduction

Protocols to transform One Shot® Mach1™-T1^R chemically competent *E. coli* are provided in this section. If you are transforming cells other than Mach1™-T1^R cells, refer to the section entitled **Transform One Shot® DH5α™-T1^R, TOP10, and TOP10F´ competent cells** (pages 16–18). If using other competent cells, follow manufacturer's instructions.

Note

The Mach1™-T1^R strain allows you to visualize colonies 8 hours after plating on ampicillin selective plates. If you are using kanamycin selection, you will need to incubate plates overnight in order to visualize colonies.

With the Mach1™-T1^R strain, you may also prepare plasmid DNA 4 hours after inoculating a single, overnight-grown colony. Note that you will get sufficient growth of transformed cells within 4 hours in ampicillin or kanamycin selective media.

Required materials

Components required but not supplied:

- The TOPO® Cloning reaction from **Perform the TOPO® Cloning reaction**, step 2 on page 12
- LB plates containing 50 µg/mL ampicillin or 50 µg/mL kanamycin
- 40 mg/ml X-gaL in dimethylformamide (DMF)
- 42°C water bath
- 37°C shaking and non-shaking incubator
- General microbiological supplies (e.g., plates, spreaders)

Components supplied with the kit:

- S.O.C. medium

Prepare for transformation

For each transformation, you will need one vial of competent cells and two selective plates.

- Equilibrate a water bath to 42°C.
- Warm the vial of S.O.C. medium from Box 2 to room temperature.
- Warm selective plates at 37°C for 30 minutes (see the following Important Note).
- Spread 40 µL of 40 mg/mL X-gal on each LB plate and incubate at 37°C until ready for use.
- Thaw *on ice* 1 vial of One Shot® cells for each transformation.

IMPORTANT!

If you are performing the rapid chemical transformation protocol or if you wish to visualize colonies within 8 hours of plating, it is essential that you pre-warm your LB plates containing 50–100 µg/mL ampicillin prior to spreading.

Continued on next page

One Shot® chemical transformation protocol

For optimal growth of Mach1™-T1^R *E. coli* cells, it is essential that selective plates are prewarmed to 37°C prior to spreading.

1. Add 2 µL of the TOPO® Cloning reaction from **Perform the TOPO® Cloning reaction**, step 2 on page 12 into a vial of One Shot® Chemically Competent *E. coli* and mix gently. **Do not mix by pipetting up and down**.
2. Incubate on ice for 5–30 minutes.

   Note: Longer incubations on ice do not seem to affect transformation efficiency. The length of the incubation is at the user's discretion.
3. Heat-shock the cells for 30 seconds at 42°C without shaking.
4. Immediately transfer the tubes to ice.
5. Add 250 µL of room temperature S.O.C. medium.
6. Cap the tube tightly and shake the tube horizontally (200 rpm) at 37°C for 1 hour.
7. Spread 10–50 µL from each transformation on a *prewarmed* selective plate. To ensure even spreading of small volumes, add 20 µL of S.O.C. medium. We recommend that you plate two different volumes to ensure that at least one plate will have well-spaced colonies.
8. Incubate plates at 37°C. If you are using ampicillin selection, visible colonies should appear within 8 hours, and blue/white screening can be performed after 12 hours. For kanamycin selection, incubate plates overnight.
9. An efficient TOPO® Cloning reaction should produce several hundred colonies. Pick ~10 white or light blue colonies for analysis (see **Analyze Positive Clones** on page 19). Do not pick dark blue colonies.

Rapid One Shot® chemical transformation protocol

An alternative protocol is provided below for rapid transformation of One Shot® Mach1™-T1^R cells. This protocol is **only** recommended for transformations using **ampicillin** selection. For more information on selecting a transformation protocol, refer to page 13.

Note: Warm LB plates containing ampicillin to 37°C prior to spreading.

1. Add **4 µL** of the TOPO® Cloning reaction from **Perform the TOPO® Cloning Reaction**, step 2, page 12 into a vial of One Shot® Chemically Competent *E. coli* and mix gently. **Do not mix by pipetting up and down**.
2. Incubate on ice for 5 minutes.
3. Spread **50 µL** of cells on a prewarmed LB plate containing 50–100 µg/mL ampicillin and incubate overnight at 37°C.

An efficient TOPO® Cloning reaction should produce several hundred colonies. Pick ~10 white or light blue colonies for analysis (see **Analyze Positive clones**, page 19). Do not pick dark blue colonies.

Transform One Shot® DH5α™-T1^R, TOP10, and TOP10F´ competent cells

Introduction

Protocols to transform One Shot® DH5α™-T1^R, TOP10, and TOP10F´ competent *E. coli* are provided in this section. Both chemical transformation and electroporation protocols are provided. If you are transforming Mach1™-T1^R cells, refer to the section entitled T**ransform One Shot® Mach1™-T1^R competent cells** (pages 14–15). If using other competent cells, follow manufacturer's instructions.

Required materials

Components required but not supplied:

- The TOPO® Cloning reaction from **Perform the TOPO® Cloning Reaction**, step 2 on page 12
- LB plates containing 50 µg/mL ampicillin or 50 µg/mL kanamycin
- 40 mg/mL X-gaL in dimethylformamide (DMF)
- 100 mM IPTG in water (for use with TOP10F´)
- 15-mL snap-cap plastic culture tubes (sterile) (electroporation only)
- 42°C water bath
- 37°C shaking and non-shaking incubator
- General microbiological supplies (e.g., plates, spreaders)

Components supplied with the kit:

- S.O.C. medium

Prepare for transformation

For each transformation, you will need one vial of competent cells and two selective plates.

- Equilibrate a water bath to 42°C (for chemical transformation) or set up your electroporator.
- Warm the vial of S.O.C. medium from Box 2 to room temperature.
- Warm selective plates at 37°C for 30 minutes (see **Important!**, page 17).
- Spread 40 µL of 40 mg/mL X-gal on each LB plate and incubate at 37°C until ready for use.
- For TOP10F´ cells, spread 40 µL of 100 mM IPTG in addition to X-gal on each LB plate and incubate at 37°C until ready for use. IPTG is required for blue/white screening.
- Thaw *on ice* 1 vial of One Shot® cells for each transformation.

Continued on next page

Transform One Shot® DH5α™-T1^R, TOP10, and TOP10F´ competent cells, continued

IMPORTANT!

If you are performing the rapid chemical transformation protocol, it is essential that you prewarm your LB plates containing 50-100 µg/ml ampicillin prior to spreading.

One Shot® chemical transformation protocol

1. Add 2 µL of the TOPO® Cloning reaction **from Perform the TOPO® Cloning reaction**, step 2 on page 12 into a vial of One Shot® Chemically Competent *E. coli* and mix gently. **Do not mix by pipetting up and down**.
2. Incubate on ice for 5–30 minutes.

   Note: Longer incubations on ice do not seem to affect transformation efficiency. The length of the incubation is at the user's discretion.
3. Heat-shock the cells for 30 seconds at 42°C without shaking.
4. Immediately transfer the tubes to ice.
5. Add 250 µL of room temperature S.O.C. medium.
6. Cap the tube tightly and shake the tube horizontally (200 rpm) at 37°C for 1 hour.
7. Spread 10–50 µL from each transformation on a prewarmed selective plate and incubate overnight at 37°C. To ensure even spreading of small volumes, add 20 µL of S.O.C. medium. We recommend that you plate two different volumes to ensure that at least one plate will have well-spaced colonies.
8. An efficient TOPO® Cloning reaction should produce several hundred colonies. Pick ~10 white or light blue colonies for analysis (see **Analyze positive clones** on page 19). Do not pick dark blue colonies.

Rapid One Shot® chemical transformation protocol

An alternative protocol is provided below for rapid transformation of One Shot® chemically competent *E. coli*. This protocol is **only** recommended for transformations using **ampicillin** selection. For more information on selecting a transformation protocol, see page 13.

Note: It is essential that LB plates containing ampicillin are pre-warmed prior to spreading.

1. Add **4 µL** of the TOPO® Cloning reaction from **Perform the TOPO® Cloning reaction**, step 2 on page 12 into a vial of One Shot® Chemically Competent *E. coli* and mix gently. **Do not mix by pipetting up and down**.
2. Incubate on ice for 5 minutes.
3. Spread **50 µL** of cells on a pre-warmed LB plate containing 50–100 µg/mL ampicillin and incubate overnight at 37°C.
4. An efficient TOPO® Cloning reaction should produce several hundred colonies. Pick ~10 white or light blue colonies for analysis (see **Analyze positive clones** on page 19). Do not pick dark blue colonies.

Continued on next page

One Shot® electroporation protocol

1. Add 18 µL of water to 6 µL of the TOPO® Cloning reaction from **Perform the TOPO® Cloning reaction**, step 2 on page 12. Mix gently.

   Note: The TOPO® Cloning reaction must be diluted in this step to prevent arcing.
2. Transfer 2 µL of the diluted TOPO® Cloning reaction (from step 1 of this procedure) into a vial of One Shot® Electrocompetent *E. coli* and mix gently. **Do not mix by pipetting up and down**.
3. Carefully transfer the solution into a 0.1-cm cuvette, avoid formation of bubbles.
4. Electroporate your samples using your own protocol and your electroporator.

   Note: If you have problems with arcing, see the following Note.
5. Immediately add 250 µL of room temperature S.O.C. medium.
6. Transfer the solution into a 15-mL snap-cap tube (e.g., Falcon) and shake for at least 1 hour at 37°C to allow expression of the antibiotic resistance genes.
7. Spread 10–50 µL from each transformation onto a pre-warmed selective plate and incubate overnight at 37°C. To ensure even spreading of small volumes, add 20 µL of S.O.C. medium. We recommend that you plate two different volumes to ensure that at least one plate will have well-spaced colonies.
8. An efficient TOPO® Cloning reaction should produce several hundred colonies. Pick ~10 white or light blue colonies for analysis (see **Analyze positive clones** on page 19). Do not pick dark blue colonies.

Note

Diluting the TOPO® Cloning Reaction brings the final concentration of NaCl and $MgCl_2$ in the TOPO® Cloning reaction to 50 mM and 2.5 mM, respectively. To prevent arcing of your samples during electroporation, the volume of cells should be 50–80 µL (for 0.1-cm cuvettes) or 100–200 µL (for 0.2-cm cuvettes).

If you experience arcing, try **one** of the following suggestions:

- Reduce the voltage normally used to charge your electroporator by 10%.
- Reduce the pulse length by reducing the load resistance to 100 ohms.
- Precipitate the TOPO® Cloning reaction and resuspend in water prior to electroporation.

Analyze transformants

Analyze positive clones

1. Take 2–6 white or light blue colonies and culture them overnight in LB medium containing 50 µg/mL ampicillin or 50 µg/mL kanamycin.

   Note: If you transformed One Shot® Mach1™-T1^R competent *E. coli*, you may inoculate overnight-grown colonies and culture them for 4 hours in **pre-warmed** LB medium containing 50 µg/mL ampicillin or 50 µg/mL kanamycin before isolating the plasmid. For optimal results, we recommend inoculating as much of a single colony as possible.
2. Isolate plasmid DNA using PureLink® Quick Plasmid Miniprep Kit (supplied with Cat. nos. K4500-02 and K4510-02 or available separately, see page 3131). The plasmid isolation protocol is included in the manual supplied with the PureLink® Quick Plasmid Miniprep Kit and is also available from www.lifetechnologies.com. Other kits for plasmid DNA purification are also suitable for use.
3. Analyze the plasmids by restriction analysis to confirm the presence and correct orientation of the insert. Use a **restriction** enzyme or a combination of enzymes that cut once in the vector and once in the insert.

Sequencing

You may sequence your construct to confirm that your gene is cloned in the correct orientation. The M13 Forward (–20) and M13 Reverse primers are included to help you sequence your insert. Refer to the maps on page 29 (pCR™2.1-TOPO® vector) or page 30 (pCR™II-TOPO® vector) for sequence surrounding the TOPO® TA Cloning® site. For the full sequence of either vector, visit www.lifetechnologies.com/support or contact Technical Support (page 32).

Continued on next page

Analyze transformants by PCR

You may wish to use PCR to directly analyze positive transformants. For PCR primers, use either the M13 Forward (–20) or the M13 Reverse primer and a primer that hybridizes within your insert. If you are using this technique for the first time, we recommend performing restriction analysis in parallel. Artifacts may be obtained because of mispriming or contaminating template. The protocol is provided below for your convenience. Other protocols are suitable.

Materials Needed

- PCR SuperMix High Fidelity (see page 31)
- Appropriate forward and reverse PCR primers (20 µM each)

Procedure

1. For each sample, aliquot 48 µL of PCR SuperMix High Fidelity into a 0.5-mL microcentrifuge tube. Add 1 µL each of the forward and reverse PCR primer.
2. Pick 10 colonies and resuspend them individually in 50 µL of the PCR cocktail from step 1 of this procedure. Don't forget to make a patch plate to preserve the colonies for further analysis.
3. Incubate the reaction for 10 minutes at 94°C to lyse the cells and inactivate nucleases.
4. Amplify for 20–30 cycles.
5. For the final extension, incubate at 72°C for 10 minutes. Store at 4°C.
6. Visualize by agarose gel electrophoresis.

IMPORTANT!

If you have problems obtaining transformants or the correct insert, perform the control reactions described on pages 22–23 to help troubleshoot your experiment.

Long-term storage

After identifying the correct clone, be sure to prepare a glycerol stock for long term storage. We recommend that you store a stock of plasmid DNA at –20°C.

1. Streak the original colony out on LB plates containing 50 µg/mL ampicillin or 50 µg/mL kanamycin.
2. Isolate a single colony and inoculate into 1–2 mL of LB containing 50 µg/mL ampicillin or kanamycin.
3. Grow until culture reaches stationary phase.
4. Mix 0.85 mL of culture with 0.15 mL of sterile glycerol and transfer to a cryovial.
5. Store at –80°C.

Optimize the TOPO® Cloning reaction

Faster subcloning

The high efficiency of TOPO® Cloning technology allows you to streamline the cloning process. If you routinely clone PCR products and wish to speed up the process, consider the following:

- Incubate the TOPO® Cloning reaction for only 30 seconds instead of 5 minutes.

  You may not obtain the highest number of colonies, but with the high efficiency of TOPO® Cloning, most of the transformants will contain your insert.

- After adding 2 µL of the TOPO® Cloning reaction to chemically competent cells, incubate on ice for only 5 minutes.

  Increasing the incubation time to 30 minutes does not significantly improve transformation efficiency.

More transformants

If you are TOPO® Cloning large PCR products, toxic genes, or cloning a pool of PCR products, you may need more transformants to obtain the clones you want. To increase the number of colonies:

- Incubate the salt-supplemented TOPO® Cloning reaction for 20–30 minutes instead of 5 minutes.

  Increasing the incubation time of the salt-supplemented TOPO® Cloning reaction allows more molecules to ligate, increasing the transformation efficiency. Addition of salt appears to prevent topoisomerase from rebinding and nicking the DNA after it has ligated the PCR product and dissociated from the DNA.

Clone dilute PCR products

To clone dilute PCR products, you may:

- Increase the amount of the PCR product
- Incubate the TOPO® Cloning reaction for 20–30 minutes
- Concentrate the PCR product

Perform the control reactions

Introduction

We recommend performing the following control TOPO® Cloning reactions the first time you use the kit to help you evaluate results. Performing the control reactions involves producing a control PCR product using the reagents included in the kit and using the PCR product directly in a TOPO® Cloning reaction.

Before starting

For each transformation, prepare two LB plates containing 50 µg/mL kanamycin.

Note: Do not use plates containing ampicillin. The control template is a plasmid that encodes ampicillin resistance. This template is carried over into the TOPO® Cloning and transformation reactions. Transformants carrying this plasmid will also be ampicillin resistant and white, resulting in an apparent increase in TOPO® Cloning efficiency, but upon analysis, colonies do not contain the desired construct.

Produce the control PCR product

1. To produce the 750 bp control PCR product, set up the following 50 µL PCR:

Control DNA Template (100 ng)	1 µL
10X PCR Buffer	5 µL
dNTP Mix	0.5 µL
Control PCR Primers (0.1 µg/µL each)	1 µL
Water	41.5 µL
Taq Polymerase (1 unit/µL)	1 µL
Total Volume	50 µL

2. Amplify using the following cycling parameters:

Step	Time	Temperature	Cycles
Initial denaturation	2 minutes	94°C	1X
Denaturation	1 minute	94°C	25X
Annealing	1 minute	55°C	
Extension	1 minute	72°C	
Final extension	7 minutes	72°C	1X

3. Remove 10 µL from the reaction and analyze by agarose gel electrophoresis. A discrete 750 bp band should be visible. Proceed to the **Control TOPO® Cloning reactions** on page 23.

Continued on next page

Perform the control reactions, continued

Control TOPO® Cloning reactions

Using the control PCR product produced on page 22 and the TOPO® vector, set up two 6 µL TOPO® Cloning reactions as described below.

1. Set up control TOPO® Cloning reactions:

Reagent	"Vector Only"	"Vector + PCR Insert"
Control PCR Product	—	1 µL
Water	4 µL	3 µL
Salt Solution	1 µL	1 µL
TOPO® vector	1 µL	1 µL
Final Volume	**6 µL**	**6 µL**

2. Incubate the reactions at room temperature for **5 minutes** and place on ice.
3. Prepare the samples for transformation:
   - For chemical transformation protocols, proceed directly to step 4.
   - For **electroporation protocols only,** dilute the TOPO® Cloning reaction 4-fold (e.g., add 18 µL of water to the 6 µL TOPO® Cloning reaction) before proceeding to step 4.
4. Transform 2 µL of each reaction into separate vials of One Shot® competent cells (pages 13–18) or equivalent competent cells.
5. Spread 10–50 µL of each transformation mix onto LB plates containing 50 µg/mL kanamycin and X-Gal (and IPTG, if using TOP10F´ cells). Be sure to plate two different volumes to ensure that at least one plate has well-spaced colonies. For plating small volumes, add 20 µL of S.O.C. medium to allow even spreading.
6. Incubate overnight at 37°C.

Analyze results

Hundreds of colonies from the vector + PCR insert reaction should be produced. 95% (+/– 4%) of these colonies will be white and 90% (or more) of these will contain the 750 bp insert when analyzed by *Eco*R I digestion and agarose gel electrophoresis. Relatively few colonies will be produced in the vector-only reaction and most of these will be dark blue. You may observe a few white colonies. This results from removal of the 3´ deoxythymidine overhangs creating a blunt-end vector. Ligation (re-joining) of the blunt ends will result in disruption of the *Lac*Zα reading frame leading to the production of white colonies.

Transformation control

Kits containing competent cells include pUC19 plasmid to check the transformation efficiency of the One Shot® competent cells. Transform with 10 pg per 50 µL of cells using the protocols on pages 13–18.

Use LB plates containing 100 µg/mL ampicillin. Just before plating the transformation mix for electrocompetent cells, dilute 10 µL of the mix with 90 µL S.O.C. medium.

Type of Cells	Volume to Plate	Transformation Efficiency
Chemically competent	10 µL + 20 µL S.O.C.	~1 × 10^9 cfu/µg DNA
Electrocompetent	20 µL (1:10 dilution)	>1 × 10^9 cfu/µg DNA

Continued on next page

Factors affecting cloning efficiency

Note that lower cloning efficiencies will result from the following variables. Most of these are easily correctable, but if you are cloning large inserts, you may not obtain the expected 95% (+/– 4%) cloning efficiency.

Variable	Solution
pH >9	Check the pH of the PCR amplification reaction and adjust with 1 M Tris-HCl, pH 8.
Incomplete extension during PCR	Be sure to include a final extension step of 7–30 minutes during PCR. Longer PCR products will need a longer extension time.
Cloning large inserts (greater than 1 kb)	Try one or all of the following: • Increase amount of insert. • Incubate the TOPO® Cloning reaction longer. • Gel-purify the insert (see page 25).
Excess (or overly dilute) PCR product	Reduce (or concentrate) the amount of PCR product.
Cloning blunt-ended fragments	Add 3´ A-overhangs to your blunt PCR product by incubating with *Taq* polymerase (page 27).
	Use the Zero Blunt® PCR Cloning Kit to clone blunt PCR products (Cat. no. K2800-20).
PCR cloning artifacts ("false positives")	TOPO® Cloning is very efficient for small fragments (less than 100 bp) present in certain PCR reactions. Gel-purify your PCR product (page 25).
PCR product does not contain sufficient 3´ A-overhangs even though you used *Taq* polymerase	Increase the final extension time to ensure all 3´ ends are adenylated. *Taq* polymerase is less efficient at adding a nontemplate 3´ A next to another A. *Taq* is most efficient at adding a nontemplate 3´ A next to a C. You may have to redesign your primers so that they contain a 5´ G instead of a 5´ T (Brownstein *et al.*, 1996).

Appendix A: Support protocols

Purify PCR products

Introduction

Smearing, multiple banding, primer-dimer artifacts, or large PCR products (greater than 1 kb) may necessitate gel purification. If you intend to purify your PCR product, be extremely careful to remove all sources of nuclease contamination. There are many protocols to isolate DNA fragments or remove oligonucleotides. Two simple protocols are described in this section.

Using the PureLink® Quick Gel Extraction Kit

The PureLink® Quick Gel Extraction Kit (page 31) allows you to rapidly purify PCR products from regular agarose gels.

1. Equilibrate a water bath or heat block to 50°C.
2. Excise the area of the gel containing the desired DNA fragment using a clean, sharp blade. Minimize the amount of surrounding agarose excised with the fragment.
3. Weigh the gel slice.
4. Add Gel Solubilization Buffer (GS1) supplied in the kit as follows:
   - For ≤2% agarose gels, place up to 400 mg gel into a sterile, 1.5-mL polypropylene tube. Divide gel slices exceeding 400 mg among additional tubes. Add 30 µL Gel Solubilization Buffer (GS1) for every 10 mg of gel.
   - For >2% agarose gels, use sterile 5-mL polypropylene tubes and add 60 µL Gel Solubilization Buffer (GS1) for every 10 mg of gel.
5. Incubate the tube at 50°C for 15 minutes. Mix every 3 minutes to ensure gel dissolution. After the gel slice appears dissolved, incubate the tube for an **additional** 5 minutes.
6. Preheat an aliquot of TE Buffer (TE) to 65–70°C
7. Place a Quick Gel Extraction Column into a Wash Tube. Pipet the mixture from step 5 of this procedure onto the column. Use 1 column per 400 mg agarose.
8. Centrifuge at >12,000 × g for 1 minute. Discard the flow-through. Place the column back into the Wash Tube.
9. *Optional:* Add 500 µL Gel Solubilization Buffer (GS1) to the column. Incubate at room temperature for 1 minute. Centrifuge at >12,000 × g for 1 minute. Discard the flow-through. Place the column back into the Wash Tube.
10. Add 700 µL Wash Buffer (W9) with ethanol (add 96–100% ethanol to the Wash Buffer according to instructions on the label of the bottle) to the column and incubate at room temperature for 5 minutes. Centrifuge at >12,000 × g for 1 minute. Discard flow-through.
11. Centrifuge the column at >12,000 × g for 1 minute to remove any residual buffer. Place the column into a 1.5-mL Recovery Tube.
12. Add 50 µL *warm* (65–70°C) TE Buffer (TE) to the center of the cartridge. Incubate at room temperature for 1 minute.
13. Centrifuge at >12,000 × g for 2 minutes. *The Recovery Tube contains the purified DNA.* Store DNA at –20°C. Discard the column.
14. Use 4 µL of the purified DNA for the TOPO® Cloning reaction.

Continued on next page

Low-melt agarose method

Note that gel purification will dilute your PCR product. Use only chemically competent cells for transformation.

1. Electrophorese all of your PCR reaction on a low-melt TAE agarose gel (0.8–1.2%).
2. Visualize the band of interest and excise the band.
3. Place the gel slice in a microcentrifuge tube and incubate the tube at 65°C until the gel slice melts.
4. Place the tube at 37°C to keep the agarose melted.
5. Use 4 µL of the melted agarose containing your PCR product in the TOPO® Cloning reaction (page 12).
6. Incubate the TOPO® Cloning reaction **at 37°C for 5–10 minutes.** This is to keep the agarose melted.
7. Transform 2–4 µL directly into competent One Shot® cells using one of the methods described on pages 13–18.

Note

Cloning efficiency may decrease with purification of the PCR product. You may wish to optimize your PCR to produce a single band.

Adding 3´ A-overhangs post-amplification

Introduction

Direct cloning of DNA amplified by proofreading polymerases into TOPO® TA Cloning® vectors is often difficult because proofreading polymerases remove the 3´ A-overhangs necessary for TA Cloning®. This section describes a simple method to clone these blunt-ended fragments.

Required materials

- *Taq* polymerase
- A heat block equilibrated to 72°C
- Phenol-chloroform (optional)
- 3 M sodium acetate (optional)
- 100% ethanol (optional)
- 80% ethanol (optional)
- TE buffer (optional)

Procedure

This is just one method for adding 3´ adenines. Other protocols may be suitable.

1. After amplification with a proofreading polymerase, place vials on ice and add 0.7–1 unit of *Taq* polymerase per tube. Mix well. It is not necessary to change the buffer. A sufficient number of PCR products will retain the 3´ A-overhangs.
2. Incubate the vials at 72°C for 8–10 minutes (do not cycle).
3. Place the vials on ice and use immediately in the TOPO® Cloning reaction.

Note: If you plan to store your sample overnight before proceeding with TOPO® Cloning, extract your sample with an equal volume of phenol-chloroform to remove the polymerases. Ethanol-precipitate the DNA and resuspend in TE buffer using the starting volume of the PCR.

Note

You may also gel-purify your PCR product after amplification with a proofreading polymerase. After purification, add *Taq* polymerase buffer, dATP, and 0.5 unit of *Taq* polymerase. Incubate the reaction for 10–15 minutes at 72°C and use in the TOPO® Cloning reaction.

Recipes

LB (Luria-Bertani) medium and plates

Composition:

1.0% Tryptone
0.5% Yeast Extract
1.0% NaCl
pH 7.0

1. For 1 liter, dissolve 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 950 mL deionized water.
2. Adjust the pH of the solution to 7.0 with NaOH and bring the volume up to 1 liter.
3. Autoclave on liquid cycle for 20 minutes at 15 psi. Allow the solution to cool to 55°C and add antibiotic if needed (50 µg/mL of either ampicillin or kanamycin).
4. Store at room temperature or at 4°C.

LB agar plates

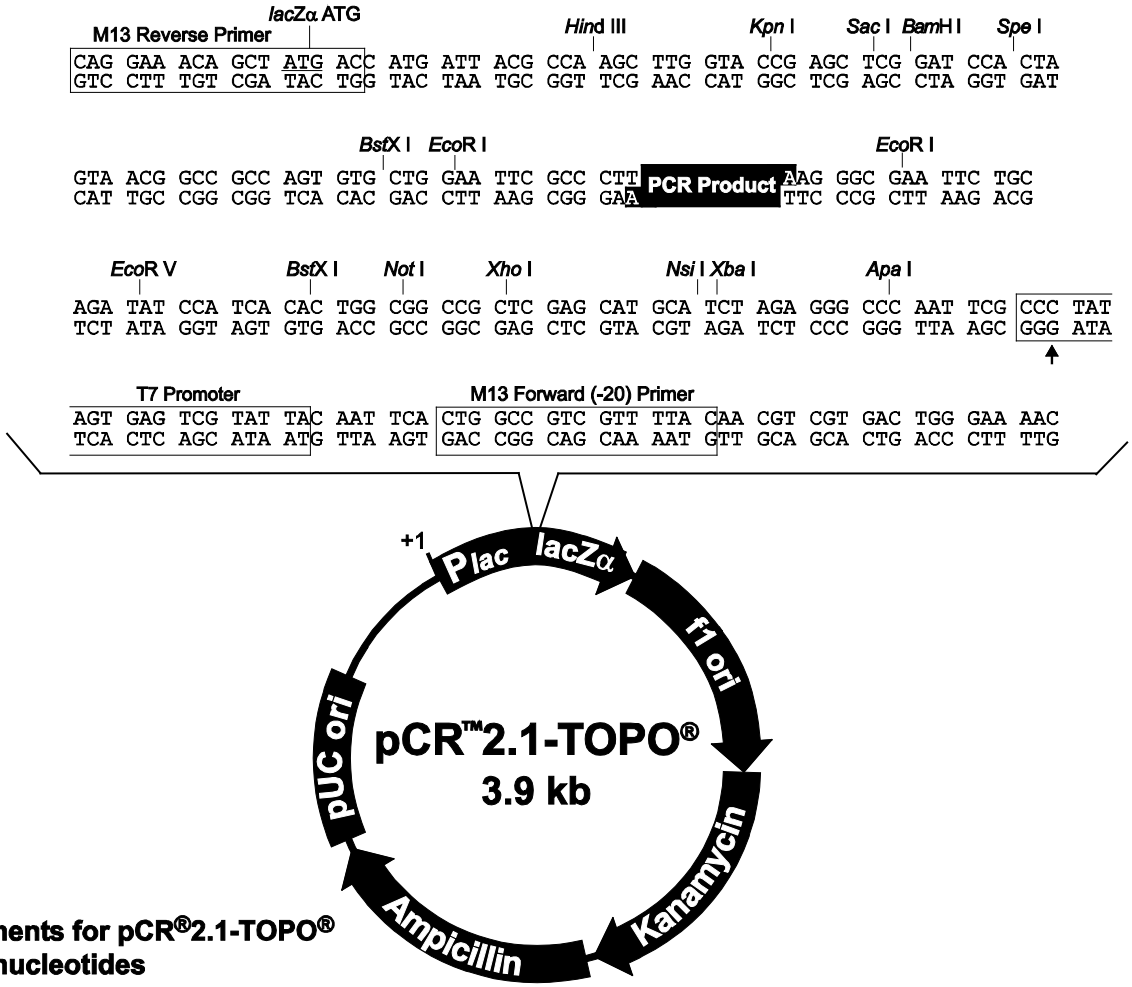
1. Prepare LB medium as above, but add 15 g/L agar before autoclaving.
2. Autoclave on liquid cycle for 20 minutes at 15 psi.
3. After autoclaving, cool to ~55°C, add antibiotic (50 µg/mL of either ampicillin or kanamycin), and pour into 10-cm plates.
4. Let harden, then invert and store at 4°C in the dark.

Appendix B: Vectors

Map of pCR™2.1-TOPO®

pCR™2.1-TOPO® map

The following map shows the features of the pCR™2.1-TOPO® vector and the sequence surrounding the TOPO® Cloning site. Restriction sites are labeled to indicate the actual cleavage site. The arrow indicates the start of transcription for T7 polymerase. The sequence of the pCR™2.1-TOPO® vector is available from www.lifetechnologies.com/support or by contacting Technical Support (page 32).


lacZα ATG
M13 Reverse Primer
HindIII
KpnI
SacI
BamHI
SpeI
CAG GAA ACA GCT ATG ACC ATG ATT ACG CCA AGC TTG GTA CCG AGC TCG GAT CCA CTA
GTC CTT TGT CGA TAC TGG TAC TAA TGC GGT TCG AAC CAT GGC TCG AGC CTA GGT GAT
BstXI
EcoRI
EcoRI
GTA ACG GCC GCC AGT GTG CTG GAA TTC GCC CTT
PCR Product
AAG GCC GAA TTC TGC
CAT TGC CGG CGG TCA CAC GAC CTT AAG CGG GAA
TTC CCG CTT AAG ACG
EcoRV
BstXI
NotI
XhoI
NsiI
XbaI
ApaI
AGA TAT CCA TCA CAC TGG CGG CCG CTC GAG CAT GCA TCT AGA GGG CCC AAT TCG CCC TAT
TCT ATA GGT AGT GTG ACC GCC GGC GAG CTC GTA CGT AGA TCT CCC GGG TTA AGC GGG ATA
T7 Promoter
M13 Forward (-20) Primer
AGT GAG TCG TAT TAC AAT TCA CTG GCC GTC GTT TTA CAA CGT CGT GAC TGG GAA AAC
TCA CTC AGC ATA ATG TTA AGT GAC CGG CAG CAA AAT GTT GCA GCA CTG ACC CTT TTG
+1
Plac
lacZα
f1 ori
pUC ori
pCR™2.1-TOPO®
3.9 kb
Kanamycin
Ampicillin

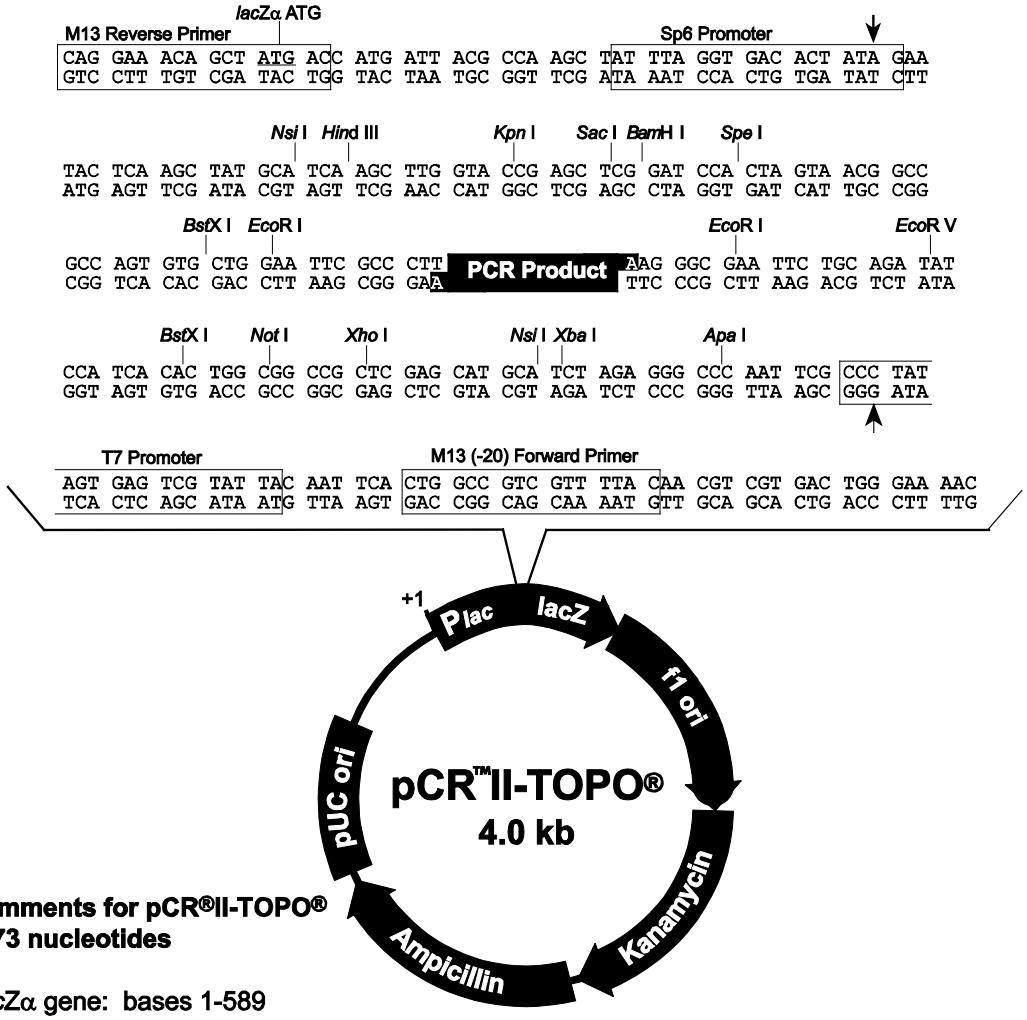
Comments for pCR®2.1-TOPO®
3931 nucleotides

*Lac*Zα fragment: bases 1-547
M13 reverse priming site: bases 205-221
Multiple cloning site: bases 234-357
T7 promoter/priming site: bases 364-383
M13 Forward (-20) priming site: bases 391-406
f1 origin: bases 548-985
Kanamycin resistance ORF: bases 1319-2113
Ampicillin resistance ORF: bases 2131-2991
pUC origin: bases 3136-3809

Map of pCR™II-TOPO®

pCR™II-TOPO® map

The following map shows the features of the pCR™II-TOPO® vector and the sequence surrounding the TOPO® Cloning site. Restriction sites are labeled to indicate the actual cleavage site. The arrows indicate the start of transcription for Sp6 and T7 polymerases. The sequence of the pCR™II-TOPO® vector is available from www.lifetechnologies.com/support or by contacting Technical Support (page 32**).**


lacZα ATG
M13 Reverse Primer
Sp6 Promoter
CAG GAA ACA GCT ATG ACC ATG ATT ACG CCA AGC TAT TTA GGT GAC ACT ATA GAA
GTC CTT TGT CGA TAC TGG TAC TAA TGC GGT TCG ATA AAT CCA CTG TGA TAT CTT
NsiI HindIII KpnI SacI BamHI SpeI
TAC TCA AGC TAT GCA TCA AGC TTG GTA CCG AGC TCG GAT CCA CTA GTA ACG GCC
ATG AGT TCG ATA CGT AGT TCG AAC CAT GGC TCG AGC CTA GGT GAT CAT TGC CGG
BstXI EcoRI EcoRI EcoRV
GCC AGT GTG CTG GAA TTC GCC CTT PCR Product AAG GGC GAA TTC TGC AGA TAT
CGG TCA CAC GAC CTT AAG CGG GAA TTC CCG CTT AAG ACG TCT ATA
BstXI NotI XhoI NsiI XbaI ApaI
CCA TCA CAC TGG CGG CCG CTC GAG CAT GCA TCT AGA GGG CCC AAT TCG CCC TAT
GGT AGT GTG ACC GCC GGC GAG CTC GTA CGT AGA TCT CCC GGG TTA AGC GGG ATA
T7 Promoter
M13 (-20) Forward Primer
AGT GAG TCG TAT TAC AAT TCA CTG GCC GTC GTT TTA CAA CGT CGT GAC TGG GAA AAC
TCA CTC AGC ATA ATG TTA AGT GAC CGG CAG CAA AAT GTT GCA GCA CTG ACC CTT TTG
+1
Plac
lacZ
f1 ori
pUC ori
pCR™II-TOPO®
4.0 kb
Kanamycin
Ampicillin

Comments for pCR®II-TOPO®
3973 nucleotides

*Lac*Zα gene: bases 1-589
M13 Reverse priming site: bases 205-221
Sp6 promoter: bases 239-256
Multiple Cloning Site: bases 269-383
T7 promoter: bases 406-425
M13 (-20) Forward priming site: bases 433-448
f1 origin: bases 590-1027
Kanamycin resistance ORF: bases 1361-2155
Ampicillin resistance ORF: bases 2173-3033
pUC origin: bases 3178-3851

Appendix C: Ordering information

Additional products

The following table lists additional products that may be used with TOPO® TA Cloning Kits. For more information, visit www.lifetechnologies.com or contact Technical Support (page 32).

Item	Quantity	Cat. no.
Taq DNA Polymerase, Native	100 units	18038-018
	500 units	18038-042
Taq DNA Polymerase, Recombinant	100 units	10342-053
	500 units	10342-020
Platinum® *Taq* DNA Polymerase High Fidelity	100 units	11304-011
PCR SuperMix High Fidelity	100 reactions	10790-020
The PCR Optimizer™ Kit	100 reactions	K1220-01
One Shot® TOP10 Chemically Competent *E. coli*	10 reactions	C4040-10
	20 reactions	C4040-03
	40 reactions	C4040-06
One Shot® TOP10 Electrocompetent *E. coli*	10 reactions	C4040-50
	20 reactions	C4040-52
One Shot® Mach1™-T1^R Chemically Competent *E. coli*	20 reactions	C8620-03
One Shot® MAX Efficiency® DH5α-T1^R Chemically Competent *E. coli*	20 reactions	12297-016
One Shot® TOP10F´ Chemically Competent *E. coli*	20 reactions	C3030-03
	40 reactions	C3030-06
Ampicillin	200 mg	11593-027
Kanamycin	5 g	11815-024
	25 g	11815-032
	100 mL (10 mg/mL)	15160-054
X-gal	100 mg	15520-034
	1 g	15520-018
IPTG	1 g	15529-019
S.O.C. Medium	10 × 10 mL	15544-034
PureLink® Quick Plasmid Miniprep Kit	50 reactions	K2100-10
PureLink® Quick Gel Extraction Kit	50 reactions	K2100-12

Appendix D: Safety

Chemical safety

WARNING!

GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING!

BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

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